PATENT COOPERATION TREATY

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INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference IN/PA-210	FOR FURTHER ACTION See Form PCT/IPEA/416	
International application No. PCT/IN2003/000296	International filing date *(day/month/year)* 03.09.2003	Priority date *(day/month/year)* 26.11.2002
International Patent Classification (IPC) or national classification and IPC A61K6/00		
Applicant DEFENCE RESEACH & DEVELOPMENT ORGANIZATION		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 6 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 2 sheets, as follows:
      ☐ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☒ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the opinion
☐	Box No. II	Priority
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
21.06.2004	13.09.2005
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Vanmontfort, D Telephone No. +49 89 2399-8457

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/IN2003/000296

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages

1-9	as published

Claims, Numbers

1-6	received on 23.06.2004 with letter of 21.06.2004

☐ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☒ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☒ the claims, Nos. 1-6
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	2-21
	No:	Claims	1,22
Inventive step (IS)	Yes:	Claims	2-6
	No:	Claims	1, 7-22
Industrial applicability (IA)	Yes:	Claims	1-22
	No:	Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

1. Section I

The amended claims 1-6, filed with the letter of 21.06.2004, introduce subject-matter which extends beyond the content of the application as filed, contrary to Article 34(2)(b) PCT.

The amendments concerned are the following:

- The feature "in animals like rabbit" in claim 1 has no basis in the application as originally filed. There is only a basis for "rabbit" (original claim 1; page 5, line 23; page 8, line 14). The feature "in animals like rabbit" is broader as "rabbit" since it includes any animal with the rabbit being a preferred embodiment.

- There is no basis whatsoever in the application as originally filed for "an agglutination reagent for rapid and early detection of typhoid". There is only basis for a process for the preparation of an agglutination reagent. Hence, claims 2-6 introduce subject-matter which extends beyond the content of the application as filed.

Therefore, the opinion has been established as if the amendments had not been made, i.e. on originally filed claims 1-22.

2. Section V

2.1 Reference is made to the following documents:

D1 US-A-4960715
D2 Lim & Choy (1988), J. Immunol. Methods, 115, 269-274

D2 was not cited in the international search report. A copy of the document is appended hereto.

2.2 The application does not meet the requirements of Article 6 PCT for the following reasons:

a) Claims 2-21 do not meet the requirements of Article 6 PCT because it is not clear to which step of claim 1 said claims are referring to. Furthermore, claims 6-21 should be made dependent from each other (as done correctly for claims 2-5) because the different dependent claims represent steps, which should be done in the proper order.

b) The feature "as substantially described and illustrated herein" of claim 22 does not meet the requirements of Article 6 PCT in that the matter for which protection is sought is not clearly defined.

2.3 Novelty & inventive step (Articles 33(2) & 33(3) PCT)

a) The subject-matter of claims 1 and 22 is not novel (Article 33(2) PCT).

D1 (examples 1 and 2; claims 1, 4 &7) discloses a process for the preparation of an agglutination reagent for rapid and early detection of typhoid, comprising the steps of (a) preparing Salmonella typhi specific antibody, (b) preparing latex particles suspension and (c) coating said latex particles with the antibody. Therefore, D1 anticipates the novelty of claims 1 and 22.

D2 discloses a latex agglutination assay for Salmonella typhi by using latex particles coated with Salmonella O-9 monoclonal immunoglobulin M (IgM) antibodies. Hence, D2 is prejudicial to the novelty of claims 1 and 22.

b) The subject-matter of claims 2-6 is novel and inventive (Articles 33(2) & 33(3) PCT) D1 or D2, which is considered to represent the closest prior art, discloses a latex agglutination assay for Salmonella typhi by using latex particles. The subject-matter of claim 2 differs in that an antibody against the flagellin gene sequence specific to Salmonella typhi is used. The problem to be solved can therefore be formulated as the provision of an alternative method to diagnose for typhoid. There is no indication in any of the available prior art documents to use an antibody against the flagellin gene sequence specific to Salmonella typhi in a method for diagnosing for typhoid. Most of the drawbacks of the conventional agglutination test for Salmonella typhi have been solved by using the above-mentioned antibody. The new agglutination

reagent provides a test with high sensitivity (93.00 %) and high specificity (98.00 %), with a diagnosis within 3 minutes after collection of serum samples and a test which enables the diagnosis of those patients who have been administered with antibiotics. Hence, the subject-matter of claim 2 is considered to involve an inventive step (Article 56 EPC). The same applies to claims 3-6.

c) Dependent claims 7-21 do not contain any features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect of inventive step (Article 33(3) PCT). The features are merely several straightforward possibilities from which the skilled person would select, in accordance with circumstances, without the exercise of inventive skill.

2.4 The following matter should also be considered:

Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in the documents D1 and D2 is not mentioned in the description, nor are these documents identified therein.

WE CLAIM:

1. A process for the preparation of an agglutination reagent for rapid and early detection of typhoid comprising :

(a) preparing antibody specific to *Salmonella typhi;*
(b) preparing latex particles suspension;
(c) coating of the said latex particles with the said antibody;

wherein the said process of preparing antibody specific to *Salmonella typhi* comprises cloning Flagellin gene sequence specific to *Salmonella typhi*, expressing the said Flagellin gene sequence by recombinant DNA technology, followed by purifying recombinant protein by affinity chromatography, raising the hyper immune sera against purified recombinant protein in animals like rabbit, separating the antibody (immunoglobulin) fraction of hyper immune sera by precipitating in ammonium sulphate, suspending in 50 mM phosphate buffer of pH 7.2 and dialyzing;

wherein the said process of preparing latex particle suspension comprises:

(i) mixing 1% carboxylated latex particles of size 0.88 to 0.90 µm and 40 mM 2-N-Morphilinoethane sulphonic acid (MES) buffer of pH 5.5 to 6.0 in a ratio of 1:1on a vortex mixer for around 60 seconds, centrifuging at 10,000 rpm for 10-12 minutes at about 4°C, followed by washing twice with 20 mM MES buffer of pH 5.5 at 10,000 rpm for 10-12 minutes at about 4°C, sonicating by a tip sonicator at about 5 watts for 60-120 seconds;

(ii) adding drop wise a freshly prepared solution of 0.1 M 1-ethyl-3 (3-dimethyl-amino propyl) carbodimide hydrochloride (EDC) in 20 mM MES buffer of pH 5.5 to the said sonicated latex particles obtained from step(i) above in a ratio of 1:1 while vortexing the suspension slowly, rotating the suspension slowly end-over-end for about 3 hours at a temperature of 20-25°C, washing thrice with 20 mM MES buffer (pH 5.5) followed by sonicating the washed suspension of latex particles by a tip sonicator for 60-120 seconds at about 5 watts;

wherein the said process of coating of the said latex particles is done by adding 0.6-1.0 mg preferably 0.8 mg per ml of the said antibody (immunoglobulins) to the said latex particle suspension, rotating the suspension end-over-end for 18-20 hours at a temperature of about 20-25°C, stopping the coating reaction by 1M glycine (pH 11.0) taken in

quantity of 0.06 ml per ml of solution of antibody coated latex particles followed by centrifugation at 10,000 rpm for 10-12 minutes at a temperature of about 4°C, washing thrice with washing buffer comprising of 50 mM glycine, pH8.5; 0.03% triton X-100 and 0.05% sodium azide, suspending in storage buffer to a final concentration of 1%, sonicating for around 60 seconds at about 5 watts and storing at 4°C.

2. An agglutination reagent for rapid and early detection of typhoid, comprising of 1% carboxylated latex particles coated with antibody specific to *Salmonella typhi*, suspended in storage buffer.
3. An agglutination reagent as claimed in claim 2 wherein the size of the said latex particles is 0.88 to 0.90 µm.
4. An agglutination reagent as claimed in claim 2 wherein the said storage buffer comprises of 50 mM glycine pH8.5, 1.0% bovine serum albumin, 0.03% triton X-100, 0.1% sodium azide and 0.01% thiomersal.
5. An agglutination reagent for rapid and early detection of typhoid as claimed in claim 2 wherein the said antibody is the immunoglobulin fraction, of the hyper immune sera raised in rabbit against the recombinant protein expressed by cloning of Flagellin gene sequence specific to *Salmonella typhi* by recombinant DNA technology, suspended in 50mM phosphate buffer.
6. A kit for rapid and early detection of typhoid comprising of 1% agglutination reagent as claimed in claim (2) suspended in storage buffer, glass slides, droppers, wooden sticks and positive & negative controls.